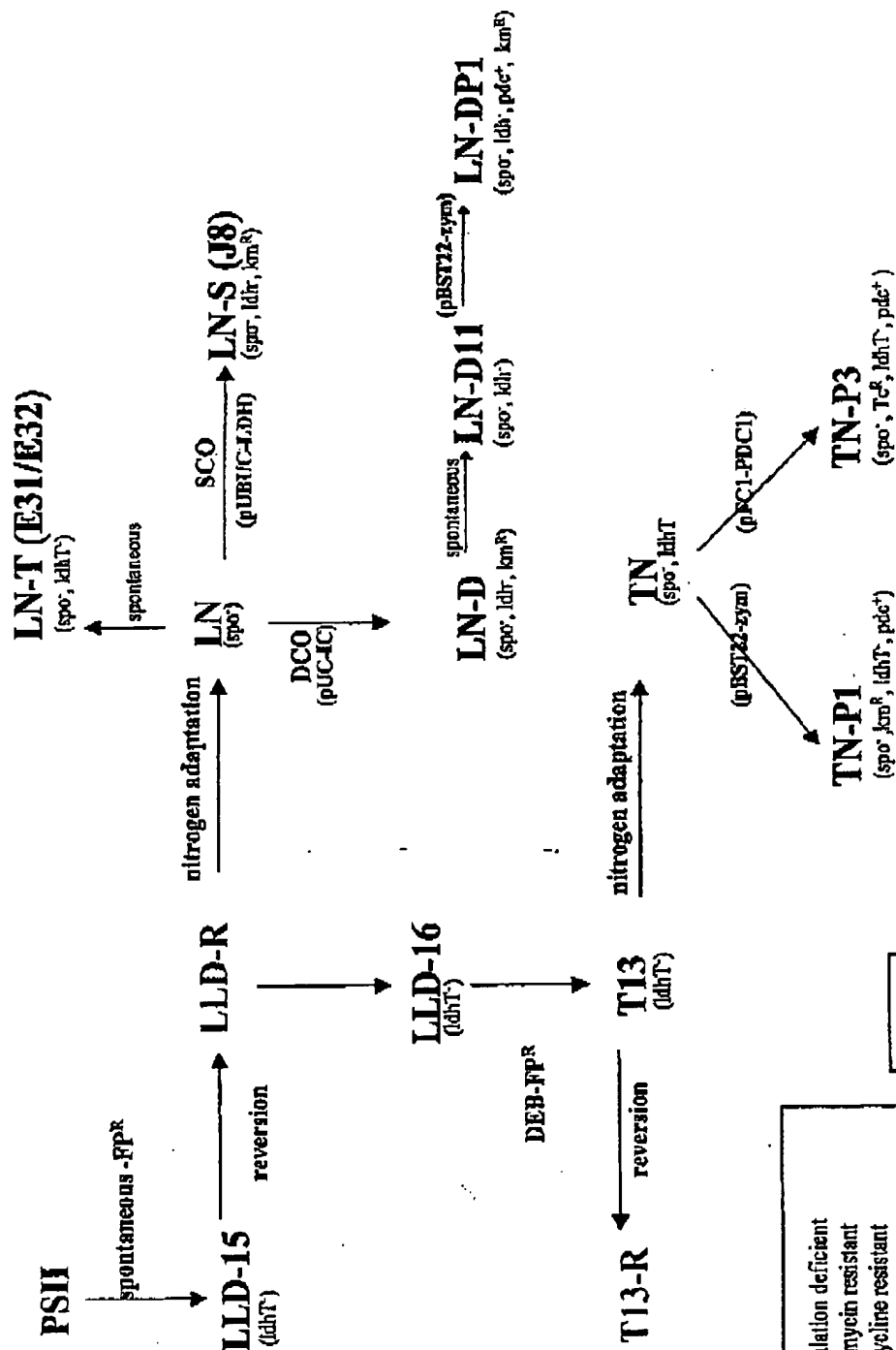


Figure 1

## Ethanol Strain Development



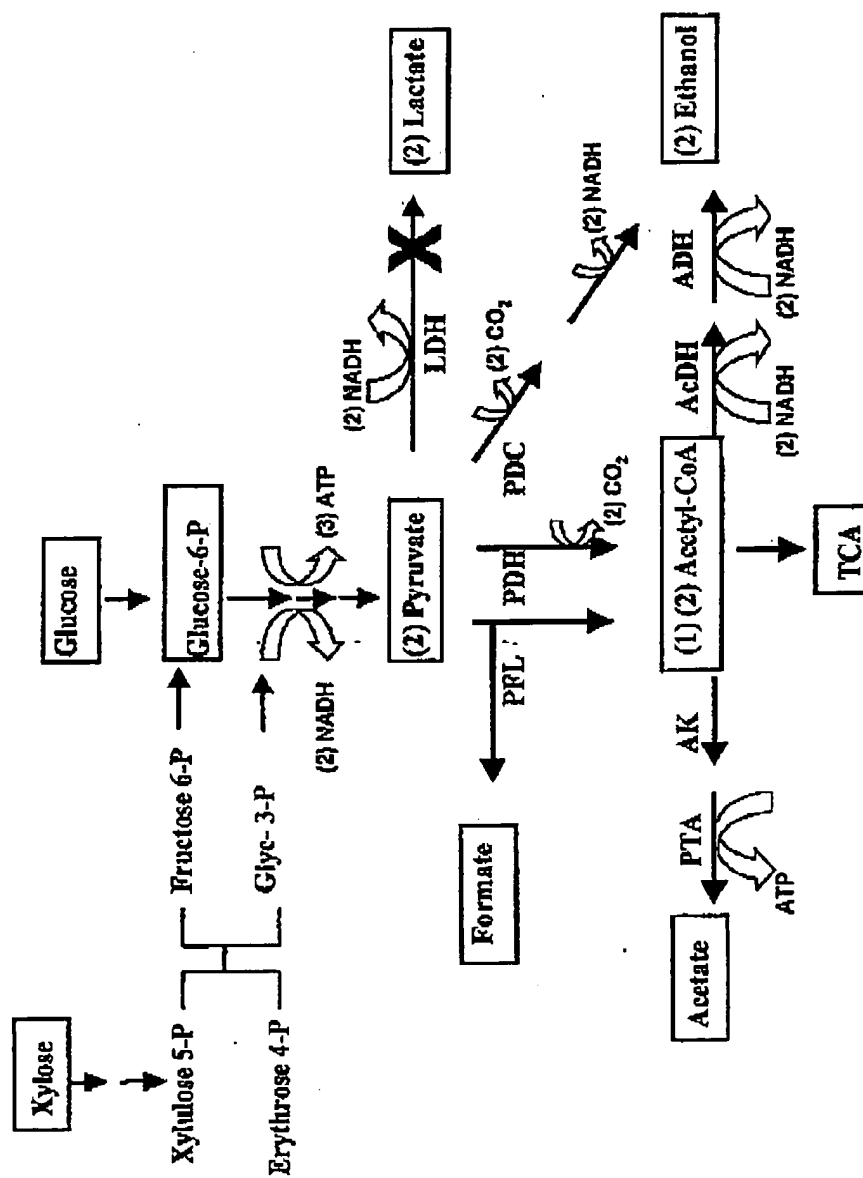
ethanol  
lactate

spo<sup>+</sup> sporulation deficient  
km<sup>R</sup> kanamycin resistant  
Tc<sup>R</sup> tetracycline resistant  
ldh<sup>-</sup> LDH recombination mutant  
ldhT<sup>-</sup> LDH transposon mutant  
pdc<sup>+</sup> pyruvate decarboxylase positive

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## Sugar Metabolism to Ethanol

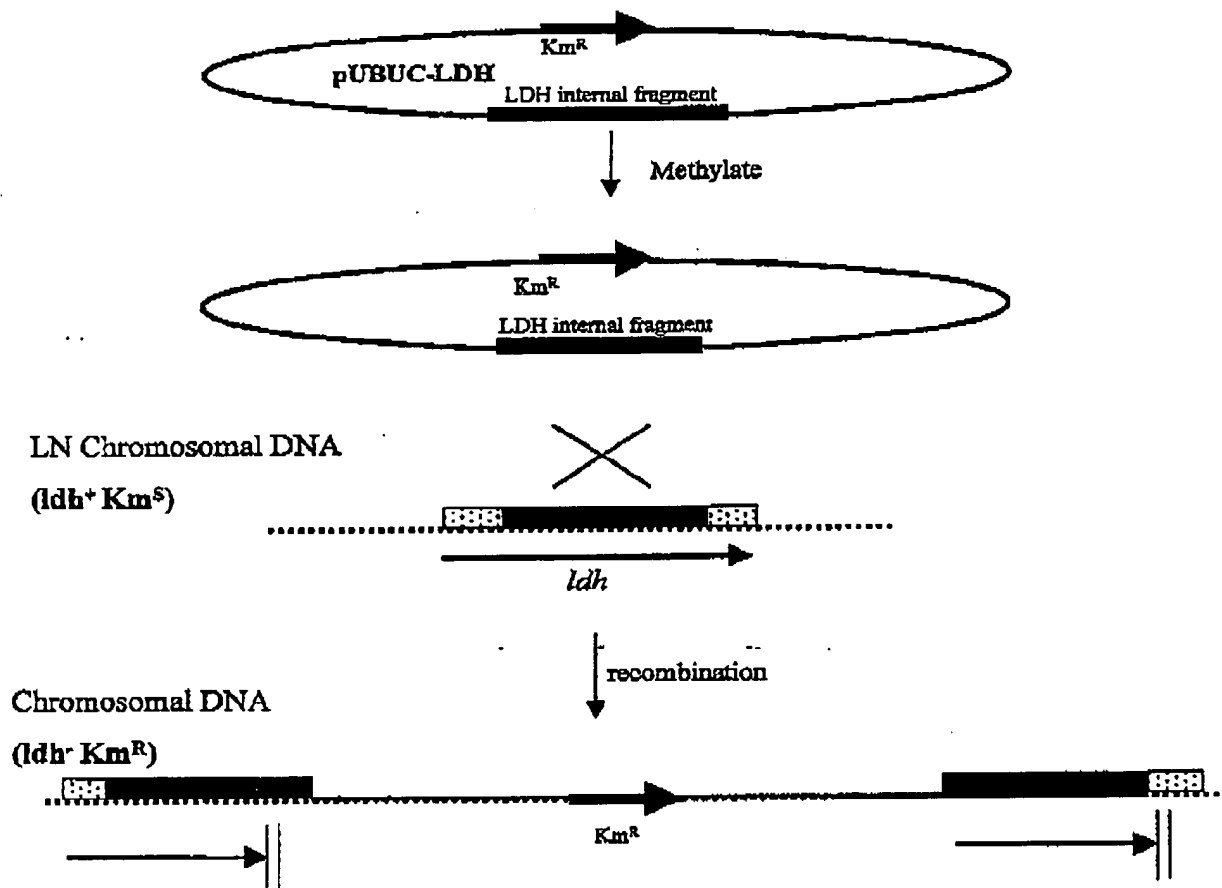
Figure 2



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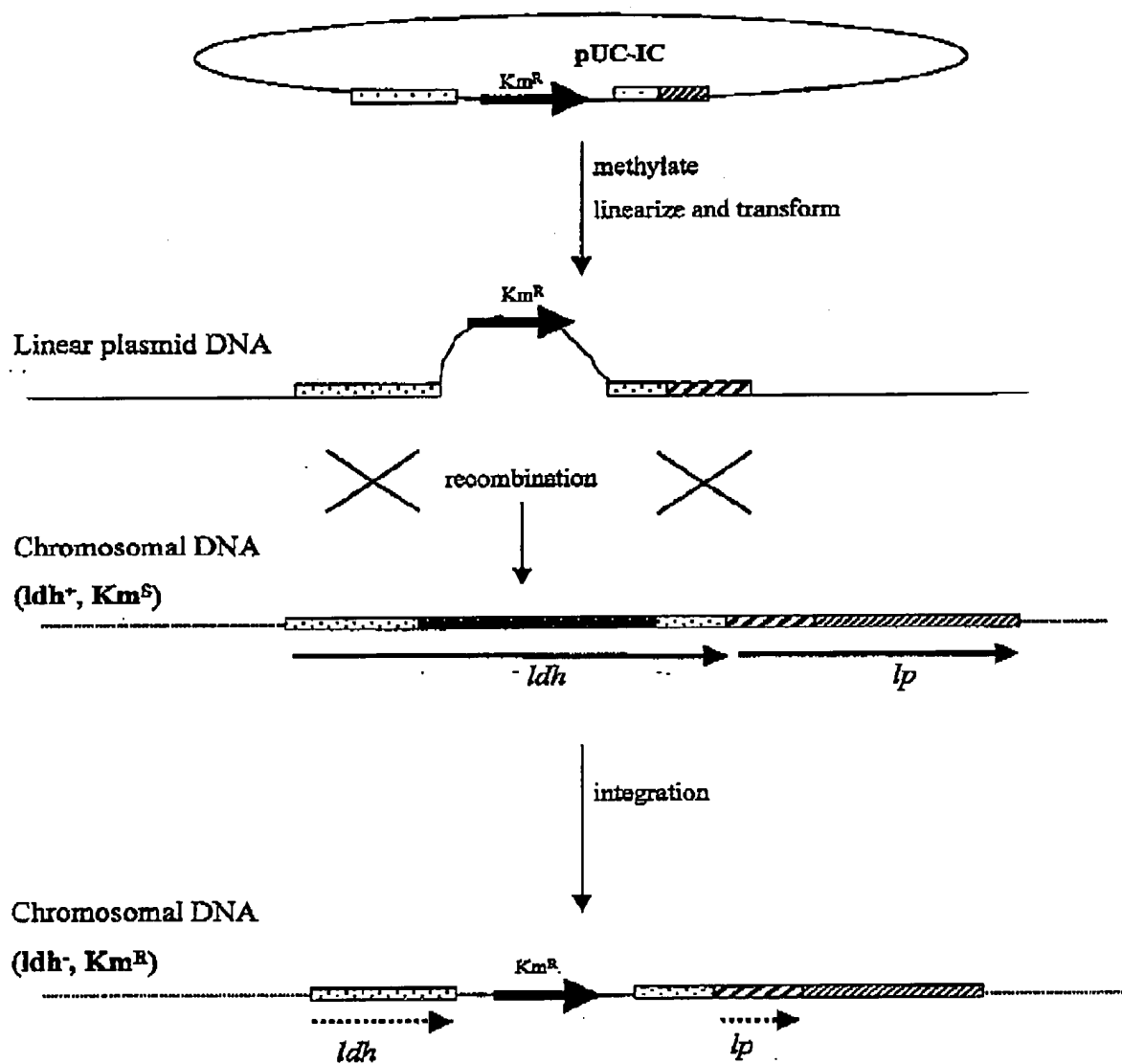
Figure 3

### LDH Gene Inactivation by Single-Crossover Recombination



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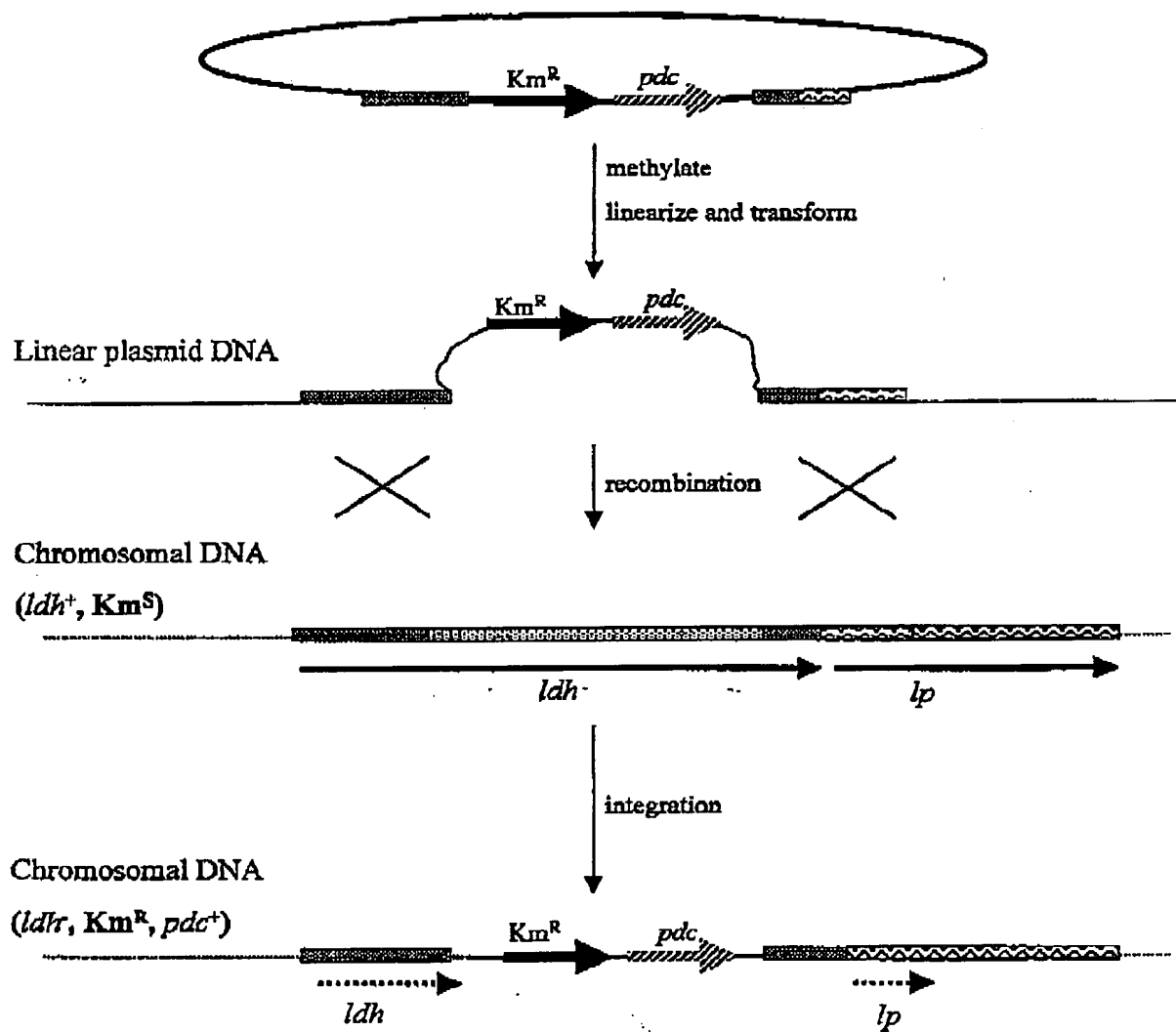
Figure 4

**LDH Gene Inactivation by Double-Crossover Recombination**

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Figure 5

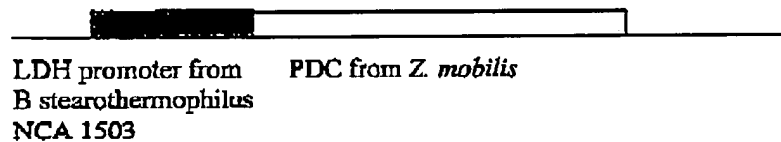
LDH Gene Inactivation and Heterologous *PDC* Gene Expression

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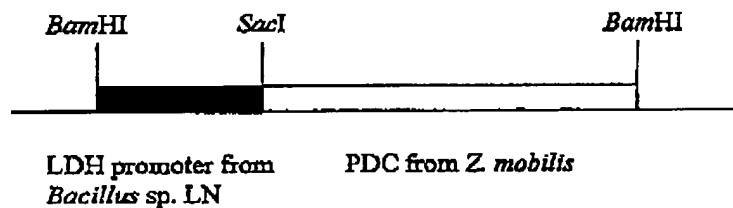
Figure 6

## Expression of PDC

### Construct 1 (cloned in pBST22)



### Construct 2 (cloned in pFC1)



### Construct 3 (cloned in pFC1)



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Figure 7. LDH promoter sequence from *Bacillus* LN

AGGGCAATCTGAAAGGAAGGGAAAATTCCTTTCCGGATTCTCCTTTTAGTTATTTTATGG - 60  
GCAGTGAATATTATATAGGCATTACGGAAATGATAATGGCAGAGTTTTTTCATTTATTAG - 120  
ACTGCTTGATGTAATTGGATGIGATGATACAAAAATAATGTTGTGTAAACAAAATGTTAA - 180  
CAAAAAAGACAAATTTCAATCATAGTTGATACTTCATAAAGATTGTGAAATAATGCACAA - 240  
TATATCAATGTATGAGCAGTTTCACAAATTCATTTTTTGGAAAGGATGACAGACAGCG'AT - 300

G

underlined: putative promoter sequences

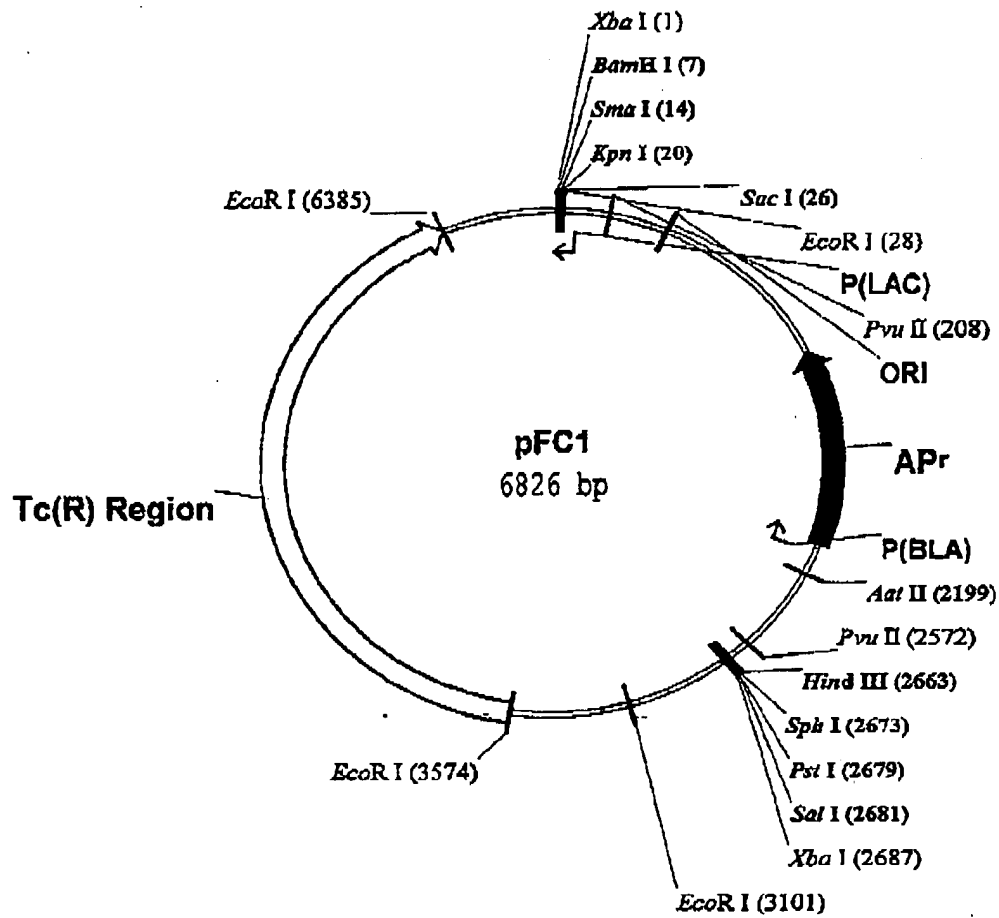
**bold**: putative ribosome binding site

\*: start codon

FOR "E" E8075260

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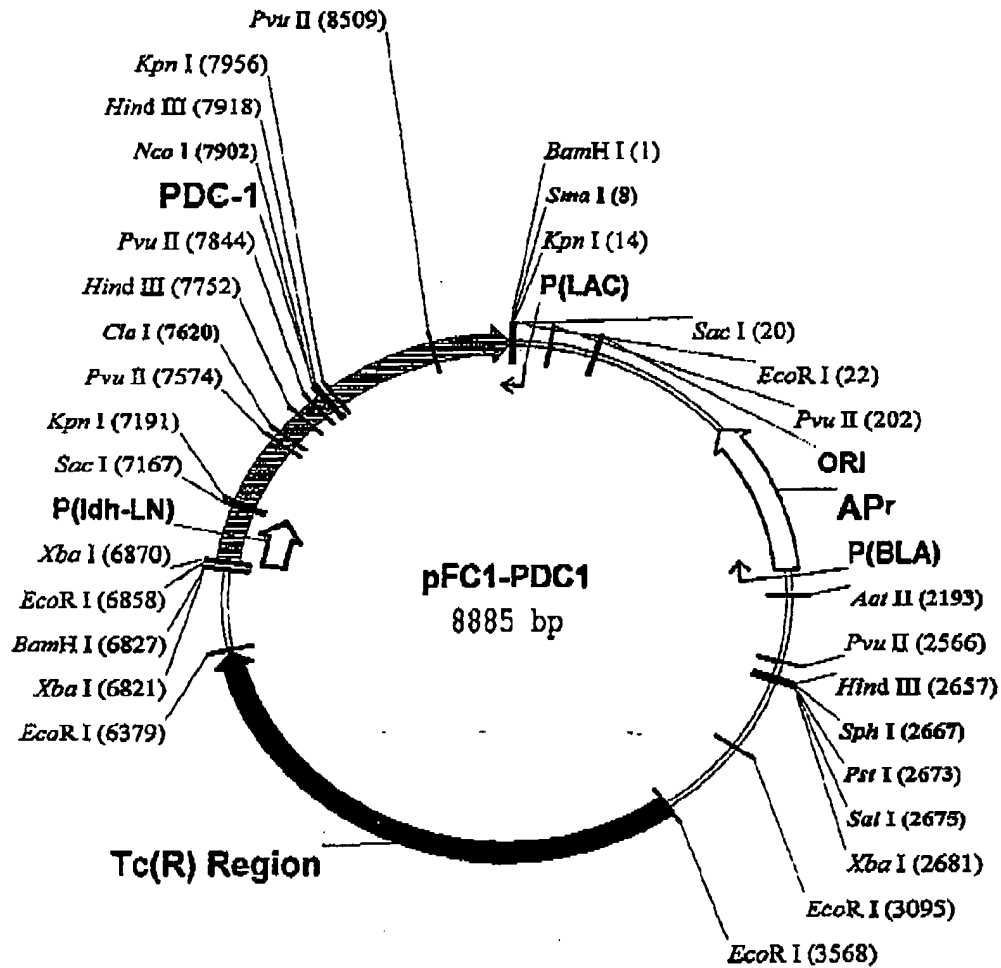
Figure 8





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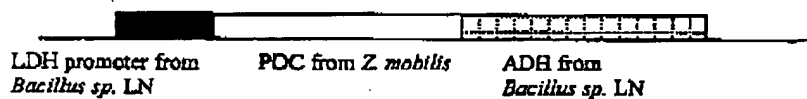
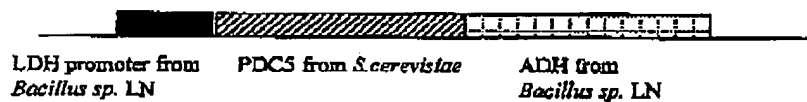
Figure 9



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Figure 10

**C nstruction of an Artificial PDC peron****Construct 4****Construct 5**

FOUO - E8075260